

WHAT IS CLAIMED:

1. A method for determining susceptibility of bacterial cells to an antibiotic comprising:

providing a test substance containing bacterial cells;

contacting the test substance with a growth medium containing an antibiotic, known to inhibit an operative enzyme of a bacterial biochemical pathway, to form a test substrate;

incubating the test substrate;

adding to the test substrate a histochemical reagent capable of generating a chromogenic compound as the result of interaction with the biochemical pathway, if the operative enzyme is not inhibited by the antibiotic; and,

observing the bacterial cells in the test substrate for the presence of the chromogenic compound.

2. The method of claim 1, further comprising:

contacting an aliquot of the test substance containing the bacterial cells with growth medium not containing the antibiotic to form a control substrate;

incubating the control substrate;

adding the histochemical reagent to the control substrate; and,

observing the bacterial cells in the control substrate for the presence of the chromogenic compound.

5           3.     The method of claim 1, wherein the bacterial cells are selected from the group consisting of *Pseudomonas*, *Eschericia*, *Streptococcus*, *Staphlococcus*, *Enterococcus*, *Enterobacteriaceae*, *Mycobacteria*, *Klebsiella* and *Haemophilis*.

10           4.     The method of claim 1, wherein the operative enzyme is selected from the group consisting of transpeptidase, carboxypeptidase, tetrahydropteroic acid synthetase and dihydrofolate reductase.

15           5.     The method of claim 1, wherein the antibiotic is selected from the group consisting of a  $\beta$ -lactam, a tetracycline, an aminoglycoside, a sulfonamide, a macrolide, a fluoroquinolone and trimethoprim antibiotic.

20           6.     The method of claim 1, wherein the antibiotic is selected from the group consisting of ampicillin, cefazolin, cephalothin, ceftazidime, gentamycin, mezlocillin, oxacillin, penicillin, piperacillin, ticarcillin and trimethoprim.

            7.     The method of claim 1, wherein the test substrate, and control substrate if used, are incubated from about 1 to about 120 minutes.

25           8.     The method of claim 7, wherein the test substrate, and the control substrate if used, are incubated from about 30 to about 90 minutes.

            9.     The method of claim 7, wherein the test substrate, and the control substrate if used, are incubated from about 10 to about 40 minutes.

10. The method of claim 1 wherein the test substance comprises a body fluid.

11. The method of claim 10, wherein the body fluid is selected from the group consisting of serum, plasma, spinal fluid, phlegm, saliva, nasal discharge, ocular discharge and pus.

12. The method of claim 1, wherein the test substance is selected from group consisting of tissue and feces.

13. The method of claim 1, wherein the chromogenic compound is observable by the naked eye.

14. The method of claim 1, wherein the chromogenic compound is observed using instrumental means.

15. The method of claim 14, wherein the instrumental means comprises a light microscope, a UV spectrophotometer or a laser scanner.

16. The method of claim 1, wherein:  
the antibiotic is trimethoprim;  
the enzyme-catalyzed biochemical pathway is a folic acid synthesis pathway;  
the test substrate is washed with pH6 phosphate buffer prior to contact with the histochemical reagent; and,  
the histochemical reagent comprises tetra nitro blue tetrazolium (TNBT), magnesium chloride, sodium azide, nicotinamide diphosphate (NADP) and dihydrofolic acid.

17. A kit for determining susceptibility of bacterial cells to one or more antibiotic(s) comprising one or more histochemical reagent(s), each of which is

capable of generating a chromogenic compound by interacting with a bacterial biochemical pathway if an operative enzyme of that pathway is not inhibited by an antibiotic.

5           18.    The kit of claim 17, further comprising one or more antibiotic(s) that is(are) known to inhibit the activity of an operative enzyme of a bacterial biochemical pathway that one or more of the histochemical reagent(s) is capable of interacting with to form a chromogenic compound if the operative enzyme is not inhibited by the antibiotic.

10

19.    The kit of claim 17, further comprising a growth medium.

20.    The kit of claim 17, further comprising a fixing agent.

15           21.    A method for determining the susceptibility of bacterial cells to a plurality of antibiotics, comprising:

providing a test substance containing bacterial cells;

20           providing a test plate having a plurality of wells, each well comprising a growth medium and a different antibiotic, wherein each antibiotic is known to inhibit an operative enzyme of a bacterial biochemical pathway;

placing an aliquot of the test substance containing the cells into each well;

25

incubating the test plate;

adding to each well a histochemical reagent, which is capable of generating a chromogenic compound as the result of interacting with a biochemical pathway, if

an operative enzyme of that pathway is not inhibited by the antibiotic in that well;  
and,

observing the bacterial cells in each well for the presence of the chromogenic  
compound.

22. A method for determining the susceptibility of bacterial cells to an  
antibiotic, comprising:

providing a test substance containing bacterial cells;

providing a test plate having a plurality of wells, each well comprising a  
growth medium and a different concentration of an antibiotic, which is known to  
inhibit an operative enzyme of a bacterial biochemical pathway;

placing an aliquot of the test substance containing the cells into each well;

incubating the test plate;

adding to each well a histochemical reagent, which is capable of  
generating a chromogenic compound as the result of interaction with the  
biochemical pathway, if the operative enzyme is not inhibited by the  
concentration of antibiotic in that well; and,

observing the bacterial cells in each well for the presence of the  
chromogenic compound.